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July 19, 2004

Dr. C. W. Jameson
National Toxicology Program Report on Carcinogens
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Dear Dr. Jameson:

As per the notice in Federal Register 69(97):28940-28944, the Triazine Network, a national coalition of farm organizations representing over forty states and thirty agricultural commodities, wishes to provide comments on the nomination of atrazine by the National Institute of Environmental Health Sciences (NIEHS) for listing in the 12th Report on Carcinogens (RoC).

Attached to this cover letter are two separate reports. The first, which follows in this same file, presents a commentary on the nomination process itself while the second, in a separate Pdf file presents a review of the available information pertaining to the assessment of the carcinogenic potential of atrazine. This review was performed by CANTOX for the Triazine Network.

First, we note that the nomination put forth by NIEHS appears to have been conducted in a vacuum, with little in the way of documentation to support the nomination, beyond citation of an International Agency for Research on Cancer (IARC) evaluation conducted on atrazine in 1999. More concerning is the fact that NIEHS has been involved in SAPs that were part of the very recent U.S. Environmental Protection Agency (EPA) (October 31, 2003) review of atrazine conducted as part of the Re-Registration Eligibility process mandated under the Federal Insecticide Fungicide and Rodenticide Act. (FIFRA). This comprehensive EPA review, which for the past 9 years has evaluated in detail the animal toxicology and epidemiology data on atrazine, concluded that atrazine was "Not likely to be carcinogenic to humans". Therefore, it seems incongruous that NIEHS, or individuals within the organization, have gone on to nominate atrazine for listing in the RoC. Also, the rationale stated to support the NIEHS nomination of atrazine, namely the IARC evaluation, in fact, does not support such a nomination. While IARC noted that there was *sufficient evidence of carcinogenicity in experimental animals* (the statement cited as NIEHS' rationale for the nomination of atrazine), the remainder of the IARC evaluation went on to state that in:

> "making its overall evaluation, the Working Group concluded that the mammary tumors associated with exposure to atrazine involve a non-DNA-reactive, hormonally mediated mechanism" and that "there is strong evidence that the mechanism by which atrazine increases the incidence of mammary gland tumors in Sprague-Dawley rats is not relevant to humans".

The above portion of the IARC review appears to have been selectively overlooked in the rationale provided for the listing of atrazine in the RoC. In essence, the IARC evaluation does not support the conclusion that atrazine can "*reasonably be anticipated to be a human carcinogen*". As a result, it does not provide a scientifically valid basis to support the nomination of atrazine for listing in the 12th RoC.

As indicated above, the second report, prepared by CANTOX HEALTH SCIENCES on behalf of the Triazine Network, presents data to support the conclusion that atrazine is not likely to be carcinogenic to humans. This is the same conclusion reached by the U.S. EPA in 2003 in their review conducted under the auspices of FIFRA. The CANTOX report's conclusion is also consistent with the classification of atrazine by the IARC in 1999 as a Group 3 chemical "The agent or mixture is not classifiable as to its carcinogenicity to humans". Ironically, at the NTP Board of Governors meeting last month, a new Group 3 IARC designation was purported to be a valid reason to propose the delisting of 2 other compounds previously listed in the RoC.

The proposed review of atrazine, should its nomination proceed to that stage, raises the specter of circumvention of due regulatory process. As the NTP is well aware, atrazine is currently the subject of an extensive review by EPA under the auspices of FIFRA. This review is near completion, with EPA issuing an Interim Re-Registration Eligibility Decision (IRED) on October 31, 2003. In reviewing the current RoC listings, we cannot identify a single agent listed in the RoC, or nominated for listing in the RoC, that has been the subject of a recent or nearly parallel review by the EPA, and which has been determined as "Not likely to be carcinogenic to humans". As a result, the nomination of atrazine raises the possibility of precedent whereby its nomination and listing (which itself would fly in the face of the scientific data) is not in accordance with the decisions of an Agency with due regulatory authority.

In conclusion, we strongly disagree with the nomination of atrazine for listing in the 12th RoC and look forward to a response from the Panel.

Respectfully,

Jere White

Jere White, Chairman

Triazine Network

COMMENTARY ON THE NOMINATION OF ATRAZINE BY NIEHS FOR LISTING IN THE 12[TH] NATIONAL TOXICOLOGY PROGRAM REPORT ON CARCINOGENS

INTRODUCTION

On May 19, 2004, a notice appeared in the Federal Register indicating that atrazine had been nominated by the National Institute of Environmental Health Sciences (NIEHS) for listing either as "*reasonably anticipated to be carcinogenic to humans*" or "*known to be carcinogenic to humans*" in the 12[th] biennial Report on Carcinogens (RoC) to be published by the National Toxicology Program in 2006. As part of the nomination process, and prior to the meeting of Report Group 1 (RG1) on the National Toxicology Program to review listing and delisting nominations, the Triazine Network has prepared this commentary on the nomination of atrazine by NIEHS for listing in the RoC.

The Triazine Network is a coalition of over 1,000 local and state agricultural associations and individual farmers located throughout the United States. The Triazine Network was formed in 1995 by the growers of over thirty commodities to provide a vehicle for the farming community to participate in the US EPA Special Review of triazine herbicides.

Our comments pertain to:

a) lack of transparency as to why NIEHS choose to nominate atrazine at this time.

b) the lack of documentation to support a nomination as either *reasonably anticipated to be carcinogenic to humans*" or "*known to be carcinogenic to humans*".

c) previous participation of the nominating agency (NIEHS) in SAPs that were part of the U.S. Environmental Protection Agency's (EPA) Re-Registration Eligibility Decision (RED) review of the carcinogenic potential of atrazine conducted for the last 9 plus years.

d) the potential for the setting of a precedent for circumvention of regulatory review of pesticides under FIFRA.

<u>LACK OF TRANSPARENCY AND DOCUMENTATION OF THE CRITERIA APPLIED BY NIEHS TO SELECT ATRAZINE FOR NOMINATION TO THE RoC</u>

Despite the vague reason provided in the Federal Register notice, the process by which atrazine was nominated for listing in the 12th RoC is not clear. Furthermore, the criteria applied by NIEHS for the selection of atrazine and its subsequent nomination have not been defined.

Criteria for nomination for inclusion in an RoC have not been clearly defined by the NTP. It is only indicated that a rationale, that may include "appropriate background information and relevant data (*e.g.*, journal articles, NTP Technical Reports, IARC listings, exposure surveys, release inventories, *etc.*)" is required to support consideration as either "a known human carcinogen" or a "reasonably anticipated to be a human carcinogen".

NIEHS' rationale for nominating atrazine was cited as the International Agency for Research on Cancer (IARC) evaluation of atrazine conducted in 1999. Certainly the citation of an IARC evaluation that indicates carcinogenic hazard to humans (*i.e.*, Group 1 or 2 classification) would qualify a nomination of a substance for consideration for listing in an RoC. However, the rationale supplied for the nomination of atrazine is in fact a misrepresentation of the IARC evaluation. The IARC evaluation of atrazine noted that while there was sufficient evidence of carcinogenicity in experimental animals (the statement cited as NIEHS' rationale for the nomination of atrazine), it went on to state:

> "[in] making its overall evaluation, the Working Group concluded that the mammary tumours associated with exposure to atrazine involve a non-DNA-reactive, hormonally mediated mechanism" and that "there is strong evidence that the mechanism by which atrazine increases the incidence of mammary gland tumours in Sprague-Dawley rats is not relevant to humans".

The IARC evaluation concluded that atrazine be classified as a Group 3 chemical "The agent or mixture is not classifiable as to its carcinogenicity to humans". Beyond the portion of the IARC review included in the rationale for nominating atrazine, the remainder of the evaluation appears to have been selectively overlooked. In essence, the IARC evaluation does not support the conclusion that atrazine can "reasonably be anticipated to be a human carcinogen". As a result, it does not provide a scientifically valid basis to support the nomination of atrazine for listing in the 12th RoC.

NOMINATION OF ATRAZINE BY NIEHS IS INCONSISTENT WITH THE RECENT EPA EVALUATION OF THE CARCINOGENIC POTENTIAL OF ATRAZINE

As stated above, atrazine has been the subject of an EPA RED review for the past 9_ years. During this time, the carcinogenic potential has been exhaustively analyzed in great detail. The EPA in 2000, and again in 2003 concluded that atrazine was not likely to be carcinogenic to humans on the basis of the fact that the mechanism by which mammary gland tumor development is promoted in female Sprague-Dawley rats was considered of no relevance to humans. This conclusion re-iterated a similar conclusion reached in the IARC evaluation of atrazine in 1999. Beyond the EPA's conclusion with respect to the mechanism of action, an independent Scientific Advisory Panel (SAP) convened by the EPA in 2000, following reviews of the available data and of extensive comments provided by the public, further validated the conclusion that atrazine was "not likely to be carcinogenic to humans".

In addition to review of the basic carcinogenicity data, and of detailed mechanistic data from animal studies, the EPA also reviewed a number of epidemiology studies that either directly or indirectly assessed the potential carcinogenic effect of atrazine. In particular, the EPA reviewed older epidemiology studies concerning potential associations between atrazine, and other pesticide exposure, and non-Hodgkin's lymphoma, as well as newer data sets involving the Agricultural Health Study and of workers employed at an atrazine manufacturing facility in St. Gabriel, Louisiana. The conclusion of the EPA was that the older epidemiology data were inadequate and provided no evidence of a casual association between atrazine exposure and human cancers. Similarly, the EPA concluded that the Agricultural Health Study provided no basis to indicate an elevated risk for cancer associated with atrazine exposure. Finally, in their review of the St. Gabriel manufacturing study, the EPA noted that an excess of prostate cancer in certain workers could largely be explained by the intensive prostate tumor antigen (PSA) testing in place at the facility during the course of the study. As a result, the conclusion was that there was not adequate evidence in the epidemiology data to indicate a causal association between human cancer risk and exposure to atrazine. As with the mechanistic data, this conclusion was scrutinized by an independent SAP convened in 2003, a panel on which members of NIEHS presided. This SAP largely echoed the conclusions of the EPA; however they indicated that the epidemiology studies, in particular the St. Gabriel and the Agricultural Health Study, could not rule out an associated between atrazine and prostate cancer. Of course this is true since epidemiology cannot prove a negative, but only test the null hypothesis.

With the preceding level of review already conducted on atrazine, and the strong scientific consensus, that atrazine should be considered as "not likely to be carcinogenic to humans", it seems incongruous that NIEHS should at this time choose to nominate atrazine for listing in an RoC. This is particularly concerning given that representatives of NIEHS have participated in SAPs, and have also had the opportunity to comment through the public docket on the EPA review conducted over the last number of years. This along with the fact that the rationale cited by NIEHS to support their nomination was in fact a misrepresentation of the IARC evaluation, raises serious doubts about the credibility of the nomination, and, moreover, is suggestive of some other motive behind the process. The NTP's RoC has been a respected and useful document, and, as it should, is intended to help safeguard public health. It would be a disservice to the NTP, the Congress, and the public if the nomination process, a process heretofore grounded in science, were to be subverted for political or ideological purposes.

PRECEDENT FOR CIRCUMVENTION OF REGULATORY PROCESSES

Beyond the misrepresentation of the IARC evaluation of atrazine to support the nomination of atrazine, a subsequent review of atrazine, should its nomination proceed to that stage, raises the specter of circumvention of due regulatory process. As we all know, atrazine is currently the subject of an extensive review by EPA under the auspices of FIFRA. This review is near completion, with EPA issuing an Interim Re-Registration Eligibility Decision (IRED) in October 31, 2003. In reviewing the current RoC listings, we cannot identify a single agent listed in the RoC, or nominated for listing in the RoC, that has been the subject of a recent or nearly parallel review by the EPA, and which has been determined as "Not likely to be carcinogenic to humans". As a result, the nomination of atrazine raises the possibility of precedent whereby its nomination and listing (which itself would fly in the face of the scientific data) is not in accordance with the decisions of an Agency with due regulatory authority. Such a situation, at least from the point of view of public perception, would place the EPA, and the regulated organizations, in this case the registrants of atrazine, in an untenable position. Despite the weight of the scientific evidence, strong public and advocacy pressure would be brought to bear on the EPA to "change" their evaluation of atrazine to reflect that of the NTP. Should this occur, the nomination process, and subsequent evaluation by the NTP and its associated subcommittees and scientific counselors would become a prime target of advocacy groups to circumvent and subvert regulatory reviews and decisions not to their liking (i.e., initiate a nomination after a favorable regulatory review has been completed). We are highly concerned that this has already started in the case of atrazine.

CONCLUSIONS

The Triazine Network would first like to thank the NTP for the opportunity to comment on the nomination of chemicals for listing or delisting in the upcoming 12th RoC.

Our comments largely stem from the fact that atrazine has already been extensively studied and evaluated, right up to the present day, by the EPA as part of the RED process mandated under FIFRA. This review clearly has concluded that atrazine is not likely to be a human carcinogen. Therefore, it is inexplicable as to why the NIEHS would nominate atrazine for listing in the RoC. Indeed, the rationale supplied to support the nomination misrepresented the conclusions of the IARC review that were, in reality, consistent with the present conclusion of the EPA.

Beyond the science that is available to support the conclusion that atrazine is not likely to be carcinogenic to humans, and hence should never have been nominated, we are further concerned that the nomination process may become a target to subvert or circumvent decisions made in regulatory reviews. Such a situation, at least from the point of view of public perception, would place the EPA, and the regulated community in an untenable position.

CANTOX
HEALTH SCIENCES INTERNATIONAL

REVIEW OF THE CARCINOGENIC POTENTIAL OF ATRAZINE IN LIGHT OF ITS NOMINATION BY NIEHS FOR LISTING IN THE 12TH NATIONAL TOXICOLOGY PROGRAM REPORT ON CARCINOGENS

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EXECUTIVE SUMMARY

The research developed to assess the carcinogenic potential of atrazine is extensive and likely greater than that conducted for any other pesticide. For that matter, the body of data available on atrazine is greater than that available for most food additives, pharmaceuticals, or most any other industrial chemical.

The animal data clearly show that atrazine has an effect on the development of commonly occurring spontaneous mammary gland tumors in the female Sprague-Dawley rat. This represents a common tumor, at a single-site, and in a single sex. There is no other evidence of a carcinogenic effect of atrazine in experimental animals, including the F344 rat and CD-1 mouse.

Atrazine is neither mutagenic or genotoxic. The mode-of-action of atrazine has been extensively documented and shown to involve delay of LH surge at the level of the hypothalamus in the female Sprague-Dawley rat leading to a persistent estrus with subsequent earlier onset of the spontaneously occurring mammary gland tumors. The pattern of reproductive ageing in the female Sprague-Dawley and related strains of rats is remarkably different between these rats and the human female. Therefore, the reproductive senescence in these rats is considered of no relevance to humans. The available data also rule out alternate modes-of-action, including potential effects of atrazine on prolactin secretion and on the activity of aromatase enzymes involved in the conversion of androgens to estrogen.

Finally, an analysis of the published epidemiology data shows no clear associations of atrazine with any site-specific cancers, including NHL. More recent data on prostate cancer, including results from an atrazine manufacturing study and the large-scale Agricultural Health Study cohort, provide no evidence of a causal association. An excess of prostate cancer cases in the manufacturing facility is largely explained by the implementation of an intensive PSA screening program during the course of the study. In summary, the human epidemiology studies do not provide any indication that atrazine is causally related to the development of any type of cancer.

With the above data evaluated and conclusions established, it must follow that atrazine be considered as "Not likely to be carcinogenic to humans". This conclusion echoes those of previous, and in general recent, reviews of the carcinogenic potential of atrazine.

The available epidemiology data are insufficient to indicate a causal relationship between atrazine exposure and increased incidence and/or mortality due to cancer. Similarly, the epidemiology data do not meet the criteria as "limited evidence of carcinogenicity in humans, which indicates that causal interpretations [are] credible...". At best the epidemiological data are inconsistent. As a result, the epidemiology data provide no basis to list atrazine in an RoC as "Known to be a human carcinogen" or as "Reasonably anticipated to be a human carcinogen". This conclusion echoes that of IARC (1999) who stated that there was *inadequate evidence* (*i.e.*, not *limited* or *sufficient evidence*) of the carcinogenicity of atrazine in humans.

The second part of the listing criteria for inclusion in an RoC as "Reasonably Anticipated to be a Human Carcinogen", refers to the strength of data from animal toxicology studies. Beyond an increase in the incidence of tumors, the criteria indicate that the data must also show one of: increases in multiple species, in multiple tissue sites, by multiple routes of exposure, or to an usual degree with regard to incidence site or type of tumor, or age at onset. While exposure to high levels (400 ppm or higher) of atrazine administration has been shown to support the development of common spontaneous mammary gland tumors in the female Sprague-Dawley rat, atrazine does not induce tumors in any other species, at any other anatomical site, or by any other route of exposure. Also, the tumor type associated with atrazine exposure in the female Sprague-Dawley rat is a common spontaneous neoplasm. Based on this analysis, there would be appear to be no scientific basis to list atrazine on the basis of the animal toxicology data.

Beyond the strict interpretation of the listing criteria which itself provides no basis for the listing of atrazine, the NTP guidelines clearly indicate that scientific judgment and mechanism of action data are to be considered. The mode-of-action of atrazine (delay of the LH surge) is considered to be of no cancer relevance to humans (IARC, 1999; SAP, 2000; U.S. EPA, 2002; PMRA, 2004). As such then, there are no other toxicology data to indicate that atrazine has a carcinogenic effect. Therefore, the animal toxicology data provide no basis for the listing of atrazine in the RoC.

Not only do the animal toxicology data provide no evidence to support listing of atrazine in the RoC, they in fact would be sufficient to nominate atrazine for ***delisting***, should it have been listed in an RoC previously. The mode-of-action data would have been sufficient for delisting much the same as the rodent forestomach tumors were discounted in the delisting of ethyl acrylate and the bladder tumors were considered not relevant to humans in the delisting of saccharin in the 10th RoC.

In conclusion, the database on atrazine, including the animal toxicology and human epidemiology data, provide no substantive scientific basis for consideration of atrazine for listing in the 12th RoC.

REVIEW OF THE CARCINOGENIC POTENTIAL OF ATRAZINE IN LIGHT OF ITS NOMINATION BY NIEHS FOR LISTING IN THE 12TH NATIONAL TOXICOLOGY PROGRAM REPORT ON CARCINOGENS

1.0 INTRODUCTION

On May 19, 2004, a notice appeared in the Federal Register indicating that atrazine had been nominated by the National Institute of Environmental Health Sciences (NIEHS) for listing either as "*reasonably anticipated to be carcinogenic to humans*" or "*known to be carcinogenic to humans*" in the 12th biennial Report on Carcinogens (RoC) to be published by the National Toxicology Program in 2006. The Federal Register notice called for submission of comments to the NTP within 60 days (*i.e.*, by July 19, 2004). As part of the this process, the Triazine Network of Garnett, KS has retained our firm, CANTOX HEALTH SCIENCES, Inc, to review and assess the available data pertaining to the carcinogenic potential of atrazine. We have reviewed the data in light of the nomination, in particular with respect to the criteria for judging whether a substance would be nominated for either listing or delisting in the upcoming RoC. The data evaluated include extensive toxicology and mechanism-of-action studies, biochemical studies, human epidemiology studies, and past reviews conducted by the International Agency for Research on Cancer (IARC) and by the U.S. Environmental Protection Agency (U.S. EPA) as part of the Re-Registration Eligibility Decision (RED) process under FIFRA. Since the data on atrazine are so extensive, what is presented here is essentially an overview of the salient points relevant to carcinogenicity assessment. The document is not intended to present a comprehensive toxicology evaluation.

The animal data, including carcinogenicity studies and genetic toxicity evaluations, are presented in Section 2.0, followed by mode-of-action data and relevant biochemical studies (Section 3.0) and the human epidemiology data (Section 4.0). The final section (5.0) presents a discussion of the data, including other recent reviews, and the conclusions with respect to the carcinogenic potential of atrazine according to the NTP criteria for listing in the RoC.

2.0 EXPERIMENTAL ANIMAL DATA

2.1 Carcinogenicity Bioassays

Atrazine has been studied in numerous standard and non-standard carcinogenicity studies in both rats (Hardisty, 1987; Pintér *et al.*, 1990; Thakur, 1992; Stevens *et al.*, 1994; Wetzel *et al.*, 1994; Morseth, 1998; Hauswirth and Wetzel, 1998; Thakur *et al.*, 1998) and mice (Innes *et al.*, 1969; Donna *et al.*, 1981, 1986; Thakur *et al.*, 1998; reviewed in Stevens *et al.*, 1999). In all of these studies, the only notable and reproducible effect, at least with respect to hyperplastic or neoplastic pathology, was the finding that atrazine, at doses of 70 ppm or more in the diet, was associated with either an increase in the incidence, or an earlier age at onset of mammary gland tumors, mainly adenocarcinomas, in female Sprague-Dawley rats.

Donna *et al.* (1981, 1986) had reported an increased incidence of lymphomas in Swiss mice dosed with atrazine; however, inappropriate routes of exposure (*e.g.*, i.p. or s.c. injection) were utilized, and reporting of these studies was incomplete.

In one of the rat studies (Pintér *et al.*, 1990), lifetime treatment of F344 rats with 750 ppm atrazine in the diet was reportedly associated with an increased incidence of mammary gland tumors in males. However, males in the 750 ppm dose group outlived the lower dose group and controls to a significant extent such that all but 2 of the mammary tumors were found after the last male control animal had died (Thakur *et al.*, 1998). As a result, the reported increase in mammary tumors in male F344 rats was not treatment-related, but an artifact of longer survival in this group (Thakur *et al.*, 1998).

The results of Donna *et al.* (1981, 1986) and Pintér *et al.* (1990) were not confirmed in subsequent standard EPA guideline, GLP-compliant 2-year oncogenicity studies in male and female CD-1 mice and in F344 rats (Thakur, 1992; Wetzel *et al.*, 1994; Thakur *et al.*, 1998; reviewed in Stevens *et al.*, 1999).

The animal data demonstrate that atrazine has an earlier onset effect on the development of commonly occurring spontaneous mammary gland tumors in the female Sprague-Dawley rat. This represents a common tumor, at a single-site, and in a single sex. There is no other evidence of a carcinogenic effect of atrazine in experimental animals. This conclusion has been echoed in the IARC (1999) and in the most recent U.S. EPA (2002) evaluations and in the EPA's interim re-registration eligibility document (2003).

2.2 Genetic Toxicity Studies

The individual genetic toxicity tests have been reviewed by IARC (1999), U.S. EPA (2002), and by the Canadian Pesticide Management Regulatory Authority (PMRA, 2004). The genotoxicity and mutagenicity test results are overwhelmingly negative, and are supportive of a general lack of effect of atrazine under most test conditions. All of the guideline studies conducted on atrazine produced by Syngenta were judged by the U.S. EPA (2002) to demonstrate no evidence of a mutagenic or genotoxic effect. The consensus view of the scientific community is that atrazine is neither mutagenic or genotoxic (Brusick, 1994; IARC, 1999; U.S. EPA, 2002; PMRA, 2004).

3.0 MODE-OF-ACTION DATA

An extensive series of short- and long-term mechanistic and biochemical studies have been conducted on atrazine (Thakur, 1991a,b, 1998; Eldridge *et al.*, 1993; Tennant *et al.*, 1994a,b; Wetzel *et al.*, 1994; Safe *et al.*, 1995; McConnell, 1995; Morseth, 1996a,b, 1998; Hauswirth and Wetzel, 1998; Stevens *et al.*, 1999), including several conducted by scientists at National Health and Environmental Effects Research Laboratory (NHEERL)/EPA (Cooper *et al.*, 1995, 1996 1998, 2000; Stoker *et al.*, 1999a,b) to elucidate the potential mechanism by which atrazine is associated with the promotion of mammary gland tumors in female Sprague-Dawley rats.

3.1 Postulated Mode-of-Action of Atrazine

First of all, atrazine apparently does not exert its action through a direct estrogenic mechanism involving binding to estrogen receptors. Atrazine treatment has failed to have any effects on estrogen responsive tissues (*i.e.*, uterus) in normal Sprague-Dawley and F344 rats, in mice, or in such tissues in ovariectomized Sprague-Dawley rats (Thakur, 1992; Wetzel *et al.*, 1994; Morseth, 1998; Hauswirth and Wetzel, 1998; Thakur *et al.*, 1998; reviewed in Stevens *et al.*, 1999). Similarly, atrazine and its metabolites have failed to produce any effect in *in vitro* assays (*i.e.*, estrogen binding) designed to assess its estrogenic potential (Tennant *et al.*, 1994a,b; Safe *et al.*, 1995; Connor *et al.*, 1996, 1998). In fact, atrazine may have slight anti-estrogenic activity (Tennant *et al.*, 1994a,b).

Investigations with intact and ovariectomized Sprague-Dawley rats have demonstrated that there was generally an increase in the incidence, or earlier onset, of mammary gland tumors at atrazine doses of 70 ppm in the diet or more (Thakur, 1991a, 1992; Eldridge *et al.*, 1993; McConnell, 1995; Morseth, 1996a,b, 1998; Stevens *et al.*, 1999). This is the result of the delay in the luteinizing hormone (LH) surge responsible for the initiation of ovulation. This process results in increased exposure of the female Sprague-Dawley rat to circulating endogenous estrogen, and subsequent estrogen-dependent stimulation of mammary gland tumor development. The basis of the proposed mode of action includes an effect of atrazine within the hypothalamus which, directly or indirectly, results in decreased secretion of norepinephrine (NE) (Cooper *et al.*, 1996, 1998, 2000) and subsequent down regulation of the release of gonadotropin releasing hormone (GnRH) which is itself dependent upon NE secretion. Since GnRH is responsible for inducing the pituitary gland to release LH, decreased gonadotropin release from the hypothalamus leads to attenuated LH release. Below some critical level, reduced circulating LH results in the failure of ovulation and the maintenance of an estrous state, with prolonged periods of elevated circulating estrogen and prolactin levels (Thakur, 1991a; Cooper *et al.*, 1995, 1996, 1998; Morseth, 1996a,b; Stevens *et al.*, 1999). Estrogen and prolactin are known to stimulate mammary gland cell proliferation and are strongly associated with mammary gland tumor development in the Sprague-Dawley rat (Eldridge *et al.*, 1998). The normally high spontaneous incidence of mammary gland tumors in this strain of rat has been attributed to the loss of hypothalamic control of LH secretion, failure of ovulation, and prolonged estrogen secretion from the ovaries (*i.e.*, prolonged estrous state associated with loss of normal estrous cycling) (Haseman *et al.*, 1984; McMartin *et al.*, 1992; Stevens *et al.*, 1999). High doses of atrazine essentially accelerate the natural reproductive ageing process in the female Sprague-Dawley rat (Wetzel *et al.*, 1994; Eldridge *et al.*, 1998; Stevens *et al.*, 1994, 1999).

3.2 Lack of Relevance to Humans of the Proposed Mode-of-Action

Atrazine has no effects on mammary gland tumorigenesis, or on tumorigenesis in any other hormone responsive organs in the other rat species (F344) tested or in CD-1 mice. Also, in ovariectomized Sprague-Dawley rats, the mechanism was prevented, and no increase in the incidence, or earlier age at onset, of mammary gland tumors was observed (Thakur, 1992; Wetzel *et al.*, 1994; McConnell, 1995; Morseth, 1998; Thakur *et al.*, 1998; Hauswirth and Wetzel, 1998; reviewed in Stevens *et al.*, 1999). The lack of effects of high doses of atrazine in the F344 rat is noteworthy since the reproductive ageing pattern in this strain is characterized by declining serum estrogen, rather than prolongation or elevation of exposures to endogenous estrogens as observed in the ageing female Sprague-Dawley rat. Also, reproductive ageing in the F344 rat, compared to the Sprague-Dawley rat, is much more similar to human female reproductive senescence in regards to declining estrogen levels with age. A summary of critical differences in the reproductive senescence patterns of female Sprague-Dawley, F344 rats, and human females is presented in Table 1.

Table 1 Comparison of the Reproductive Senescence of Female Humans, F344 Rats, and Sprague-Dawley Rats

Parameter	Human Female	Female F344 Rat	Female S-D Rat
Time of onset (percent of lifetime)	about 70%	60-70%	30-40%
Site of action	ovaries	hypothalamus	hypothalamus
Mechanism of occurrence	follicle depletion	loss of prolactin control	impaired LH/FSH control
Overall LH surge	maintained	maintained	lost or greatly reduced
Cycle pattern	anestrus	pseudopregnancy	persistent estrus
Estrogen secretion	decreased	decreased	increased
Estrogen: progesterone	no change	decreased	increased
Prolactin secretion	low	episodic	continuous

The mechanistic data show a coherence of effects with dose-response, and a temporal pattern of effects that correlate well with the sequence of key events required in the postulated mode of action. In total, the available mechanistic data, along with other data that exclude genotoxicity and direct estrogenic activity as possible mode(s) of action, lead to the conclusion that the proposed mode of action is the only plausible biochemical explanation. The effect of atrazine, being limited to a single sex and strain of rat, with a different reproductive senescence pattern than is seen in humans strongly indicates that this mechanism is unlikely to be operative in humans.

3.3 Evaluation of Alternative or Other Possible Modes of Action

Over the past few years, there has been speculation put forth relating to potential alternative modes-of-action for atrazine and/or for modes of action that could involve increased risk for hormonally cancers. These include: a) the potential for atrazine to alter the hormonal milieu such that there may be an increased cancer risk in several endocrinologically active/responsive tissues in humans, including the mammary gland, b) data from human disease conditions in which the LH surge and/or estrogen levels are perturbed, c) potential effects on aromatase enzymes (Sanderson *et al.*, 2000, 2002), enzymes that are involved in the conversion of androgens to estrogen, and d) effects of atrazine on the development of prostatitis in neonatal rat pups (Stoker *et al.*, 1999a,b). Since all of these effects are endocrine-related, they were reviewed for their potential to alter hormonal status and to subsequently increase the risk for hormonally dependent cancers in humans

3.3.1 Effects of Atrazine on the Hormonal Milieu in the Female Sprague-Dawley Rat - Extrapolation to Humans?

As stated above, there previously existed speculation on the potential for atrazine to generally alter hormonal status such that there may be an increased cancer risk in endocrinologically active/responsive tissues in humans. This speculation was based on the assumption that cellular processes underlying the functioning of the hypothalamic-pituitary-ovarian axis are highly conserved across species and upon studies of human disease conditions in which there is disruption of normal ovulation. First off, species- and strain-specific differences in the responsiveness to hormonal or pharmacologically active substances are particularly evident in the scientific literature. Examples of rodent-specific responses to pharmacologically or endocrinologically active substances include: a) the unique pharmacologic response of rats (Claman, 1972, 1975) to corticosteroids resulting in tumor development; b) Leydig cell tumors and pheochromocytomas of ageing rats induced by certain polyols (McClain, 1994; Lynch *et al.*, 1996; Clegg *et al.*, 1997); c) thyroid follicular cell tumor response of rats to inhibitors of thyroid hormone synthesis or which increase the rate of metabolism of triiodothyronine/thyroxine (Alison *et al.*, 1994; McClain, 1994); d) uterine endometrial carcinomas of rats induced by dopamine agonists such as bromocriptine (Alison *et al.*, 1994; Monro, 1994); and, e) mesovarian leiomyomas in rats resulting from exposure to certain β2-agonists (Alison *et al.*, 1994; Monro, 1994). For many of these examples, there is widespread scientific consensus that the modes-of-action involved are likely of little relevance to human cancer risk (Alison *et al.*, 1994; McClain, 1994; Monro, 1994) despite the fact that the physiology and cellular biochemical processes of the organ systems are qualitatively similar between rodents and humans. The EPA SAP (2000) on atrazine's mode-of-action concluded that the mode-of-action for mammary gland tumors in the Sprague-Dawley rat is not relevant to humans.

3.3.2 Ovarian Diseases in Humans in Relation to Atrazine's Potential Effects on Hormone Status

A review of 2 separate human disease conditions, hypothalamic amenorrhea and polycystic ovary syndrome, also serves to underscore the lack of relevance of the mode-of-action of atrazine identified in female Sprague-Dawley rats.

3.3.2.1 Hyothalamic Amenorrhea

Hypothalamic amenorrhea is characterized by a failure of ovulation sometimes associated with severe emotional stress, heavy exercise and oral contraceptive use (Reame *et al.*, 1985). In this condition, serum estrogen and LH levels are low to near normal and there is no associated pathology in the ovaries or pituitary gland. Citing the reduced LH levels and failure of ovulation, once could compare this to the effects of atrazine in female Sprague-Dawley rats. Also, since epidemiological data (Schacter and Shoham, 1994) are suggestive of a link between hypothalamic amenorrhea and endometrial hyperplasia, a possible precursor of endometrial cancer, one could speculate that the mode-of-action for atrazine could influence human cancer risks at sites beyond the breast. However, such inferences are speculative in nature and do not account for several important differences between human hypothalamic amenorrhea and effects of atrazine in female Sprague-Dawley rats. First, estrogen levels in human hypothalamic amenorrhea are low to normal, not continuously elevated. Unlike in atrazine-treated female Sprague-Dawley rats, in the human condition the ovaries are quiescent and not hyperfunctional. Second, LH in the human condition is often near normal, while LH release in atrazine treated female Sprague-Dawley rats is significantly attenuated. Finally, no association between hypothalamic amenorrhea and cancer of the breast or any of other endocrinologically responsive organs has been reported in the scientific literature. Rather, exercise, a risk factor for development of hypothalamic amenorrhea, is associated with a reduced risk for breast cancer (Bernstein *et al.*, 1994). Additional details of the dissimilarities between effects associated with human hypothalamic amenorrhea and the effects of atrazine in female Sprague-Dawley rats are provided in Breckenridge *et al.* (2000).

3.3.2.2 Polycystic Ovary Syndrome

The second human disease state, polycystic ovary syndrome, is characterized by irregular menstrual cycles, often with amenorrhea, infertility, cystic ovaries, and persistent failure of ovulation accompanied by continued estrogen stimulation (Herschlag and Peterson, 1996;

Schildkraut *et al.*, 1996). While epidemiological associations between this condition and increased risks for endometrial and ovarian cancers in humans exist, most of the features of polycystic ovary syndrome, such as increased LH secretion from the pituitary gland and increased synthesis and conversion of androgens to estrogens, are markedly different than the effects of atrazine on female Sprague-Dawley rats (Eldridge, 2000).

Data on hypothalamic amenorrhea and polycystic ovary syndrome provide no evidence to indicate that the mode-of-action identified for atrazine in Sprague-Dawley rats could be operative in humans. In fact, the nature of these 2 conditions demonstrate that the mode-of-action would be highly unlikely to occur in humans due to the physiological differences in the reproductive senescence patterns between human females and the female Sprague-Dawley rat.

3.3.3 Effects of Atrazine on the Activity of Aromatase Enzymes

In vitro, atrazine was reported to induce the activity of aromatase enzyme in human adrenocortical carcinoma cells (Sanderson *et al.*, 2000, 2002). Subsequently, Sanderson *et al.* (2001) tested the ability of atrazine and its metabolites to induce aromatase activity *in vitro* in 3 human cancer cell lines (adrenocortical carcinoma, placental choriocarcinoma, and breast cancer) and in the liver cells of carp. Weak induction (*i.e.*, generally 3.0-fold or less) of the enzyme (CYP19) was reported in the adrenocortical and choriocarcinoma cell lines exposed to atrazine (10 to 30 μM for 24 hours), but not in the breast cancer or fish liver cell lines. In the carp liver cells, atrazine failed to induce vitellogenin production, a marker for the potential aromatization of testosterone and methyl testosterone to 17β-estradiol. Sanderson and colleagues (Heneweer *et al.*, 2004) recently reported that atrazine did not induce aromatase activity in a rat Leydig cell carcinoma line, but stated that this cell line maybe inappropriate for the study of induction of this enzyme. Keller and McClellan-Green (2003), using atrazine at concentrations of 0.1 to 30 μM, reported induction of aromatase activity *in vitro* in an immortal sea turtle cell line, although without any clear dose-response relationship.

In contrast to these conflicting *in vitro* data, in rat pups exposed to atrazine *in vivo*, the expression of aromatase enzymes was actually found to be decreased (Rayner *et al.*, 2004). Likewise, studies that have evaluated the effects of atrazine, at concentrations of up to 25 μg/L on aromatase activity in frogs (*Xenopus laevis*) in relation to purported endocrinological effects in this species, found no evidence of aromatase induction in either brain tissue or in the gonads (Hecker *et al.*, 2003; Villeneuve *et al.*, 2003). Since the increased estrogen levels occur in the female Sprague-Dawley rat following exposure to atrazine at doses sufficient to induce anovulation, the role of atrazine in the induction of aromatase *in vivo* is questionable. In any case, given the conflicting nature of the results reported with respect to aromatase induction and the lack of any consistent experimental evidence that atrazine alters androgen production or otherwise disrupts androgen-receptor complex formation, the studies reporting that atrazine induces aromatase activity do not provide evidence to suggest that atrazine may have carcinogenic potential *via* this mechanism.

3.3.4 Atrazine and the Development of Prostatitis in Neonatal Rat Pups

Stoker *et al.*, 1999a,b report that high doses (on the order of 25 mg/kg body weight/day) of maternally administered atrazine resulted in inflammation of the dorsolateral prostate of rat pups. The effect was shown to likely be the result of the suppression of prolactin secretion in dams. Suppression of maternal prolactin secretion during postnatal days 1 though 4 was further shown to cause a delay in the development of the tuberoinfundibular dopamine (TIDFA) neurons in the hypothalamus of the male rat pup. This leads to persistent hyperprolactinemia in the rat pups with subsequent development of prostatitis (Stoker *et al.*, 1999a). Prolactin is known to be a trophic hormone for the prostate (Negro-Vilar *et al.*, 1977) and to be associated with both hypertrophy and inflammation of the dorsolateral prostate in rats (Stoker *et al.*, 1999b). Essentially, attenuation of maternal prolactin at a specific time point resulted in the hypersecretion of prolactin in male rat pups leading to inflammation of the prostate (Stoker *et al.*, 1999a,b).

The above data on the induction of prostatitis in male rat pups cannot be "extrapolated" to speculate on the presence of a possible mode-of-action whereby atrazine could be carcinogenic by a means other than through the attenuation of the LH surge in the female Sprague-Dawley rat. First, the numerous (*i.e.*, more than 20) studies conducted on atrazine in rats, mice, and dogs show no evidence of any consistent and reproducible effect of atrazine on the prostate. Second, in adult male rats, atrazine has been shown to suppress prolactin secretion. As a result, in mature rats, the decreased secretion of the trophic substance prolactin in response to atrazine would suggest a decrease in hypertrophy, growth, and/or inflammation of the prostate gland. Finally, there is no consistent evidence from human epidemiology studies that prostatitis is causally related to the development of prostate cancer (DeMarzo *et al.*, 1999). Overall, the animal data do not support a mechanism for atrazine contributing to the onset, development, or promotion of prostate cancer. In addition, the mode-of-action identified by Stoker, Cooper and associates (Stoker *et al.*, 1999a,b; Cooper *et al.*, 2000) may be unique to the rat.

3.4 Summary and Discussion of the Mode-of-Action Data

In summary, the mode-of-action of atrazine, namely delay in the LH surge at the level of the hypothalamus in the female Sprague-Dawley rat leading to a persistent estrogenic state, with subsequent promotion of spontaneously occurring mammary gland tumors, has been well characterized and documented. Although the mechanism is not known down to the exact molecular level (few "modes-of-action" can be characterized to this level), it is the only biologically plausible mechanism to explain the empirical data. This mode-of-action, due to the existence of significant differences in reproductive ageing patterns between the female Sprague-Dawley rat and humans, is considered of no relevance to humans. Finally, a review of potential alternate modes-of-action (*i.e.*, comparison to human disease conditions, suggestions of increased aromatase activity, and extrapolation of the prolactin-prostate response in neonatal rats) to either explain the mammary tumors in female Sprague-Dawley rats, or to speculate on influence of other cancers in humans (*i.e.*, ovarian and prostate), clearly dismisses these as possible candidates. They either lack consistency with the known animal toxicology data, or are inconsistent with human physiological and reproductive processes.

4.0 EPIDEMIOLOGY STUDIES

There exist a number of epidemiology studies that have evaluated potential exposure to atrazine, either alone or in combination with other pesticides, on the incidence of various human cancers, notably non-Hodgkin's lymphoma, and cancers of the breast, ovary, and prostate.

4.1 Older Studies of Farm Populations and the Incidence of Various Cancers

Loosli (1995), Neuberger (1996), and Sathiakumar and Delzell (1997) examined existing case-control studies on the relationship between cancer incidence and exposure to triazine herbicides. Among the studies reviewed were 9 of farm populations in the U.S. Midwest (Hoar *et al.*, 1985, 1986; Hoar Zahm *et al.*, 1988, 1993a,b; Brown *et al.*, 1990, 1993; Burmeister, 1990; Cantor *et al.*, 1992).

The 9 farm-related studies were of similar design, evaluating relationships between various agricultural chemicals and cancer. None was designed to specifically address exposure to atrazine by itself, or to any other specific triazine herbicide. Many of the studies also reported on potential exposure to phenoxy acid herbicides and cancer incidence. Five of these studies reported possible relationships between triazine herbicide exposure, and non-Hodgkin's lymphoma (Hoar *et al.*, 1986; Hoar Zahm *et al.*, 1988, 1993a,b; Cantor *et al.*, 1992). The odds ratios for non-Hodgkin's lymphoma were marginally greater than unity (range of 1.2 to 2.5) for use of any triazine herbicide or of atrazine specifically (Neuberger, 1996; Sathiakumar and Delzell, 1997). Several factors limit the interpretation of these values. These included (1) the small numbers of incident cases in most of the studies; (2) the self-report nature of the exposure data; (3) the high percentage of subjects requiring proxy interviews (30 to 50%); (4) the use of only "non-farmers" as a reference group; (5) the lack of accounting for multiple chemical exposures from other occupations; and (6) except for one study, a lack of detailed exposure duration and time since initial exposure (Neuberger, 1996; Sathiakumar and Delzell, 1997). In one study (Hoar Zahm *et al.*, 1993a), data from other studies of U.S. mid-western farm populations (Hoar *et al.*, 1986; Hoar Zahm *et al.*, 1988; Cantor *et al.*, 1992) were pooled for increased statistical power. This produced a weak positive association between non-Hodgkin's lymphoma and atrazine exposure. However, upon adjustment for confounders, the odds ratio was reduced to near unity (Neuberger, 1996; Sathiakumar and Delzell, 1997). Moreover, there was no association among subjects with long interval from initial exposure, and no clear or consistent dose-response relationship was evident for duration or frequency of use.

Neuberger (1996) and Sathiakumar and Delzell (1997) both concluded that all of the available epidemiological studies of farm populations provided no substantive evidence to show a causal relationship between atrazine (triazine) exposure and non-Hodgkin's lymphoma.

More recently, Schroeder *et al.* (2001) reported a weak, but significant, association [Odds Ratio (OR)=1.7 with CI of 1.0-2.8] between atrazine exposure in Iowa and Minnesota farmers and risk for the t(14;18)-negative subtype of NHL. No association was reported for the t(14;18)-positive NHL subtype. However, subsequently, Deroos *et al.* (2003) published an article citing similar OR for atrazine exposure and NHL, yet concluding that "Reported use of several individual pesticides was associated with increased NHL incidence [but that] limitations of [their] data hinder the inferences [that] we can make regarding specific pesticides". As noted by the U.S. EPA, epidemiology studies with borderline significance, especially "ecological" studies, increase the likelihood that a given finding occurred by chance or was influenced by the presence of some unknown, or poorly controlled confounding variable.

4.2 Atrazine and the Incidence of Breast Cancer in Kentucky Women

Kettles *et al.* (1997) assessed breast cancer incidence in Kentucky women with known or suspected triazine herbicide exposure based upon water contamination reports, corn crop production, and pesticide use data. This ecological study evaluated breast cancer incidence in counties with "low", "medium", or "high" exposure levels as defined by the surrogate measures of triazine exposure. A Poisson regression analysis was performed to control for age, race, age at first live birth, income, and education level. The authors reported an increased OR for the "medium" and "high" exposure categories of 1.14 (CI=1.08-1.19) and 1.2 (CI=1.13-1.28), respectively. The authors cautioned that causality could not be established due to the inherent weaknesses of ecological study design. This conclusion is consistent with the U.S. EPA (2003) description of the limitation of these types of analyses. Moreover, the very small ORs reported by these authors further weaken the possibility of a causal effect. More recently, a follow-up study on the same population conducted by Hopenhayn-Rich *et al.* (2002) failed to show an association of atrazine exposure with an increased risk for either breast or ovarian cancer. In fact, a slight protective effect was reported.

4.3 Atrazine and Prostate Cancer

Recently, atrazine has been the subject of research interest with respect to potential associations with increased risks for prostate cancer. Data are derived from 3 groups or types of studies. First are the ongoing epidemiological analyses of workers employed at an atrazine manufacturing plant study in St. Gabriel, Louisiana (Delzell *et al.*, 2001; MacLennan *et al.*, 2002, 2003; Hessel *et al.*, 2004). Secondly, there are the data available from the Agricultural Health Study cohort of pesticide applicators in Iowa and North Carolina (Alavanja *et al.*, 2003), and thirdly, evaluations of cancer incidence in California counties (Mills, 1998; Mills and Yang, 2003).

First, in the manufacturing workers' study (St. Gabriel, Louisiana), a significant excess of prostate cancer (17) *versus* the number expected for that population (6.7-9.6) (Standardized Incidence Ratio of 178 to 255) was reported. In addition, 5 cases were identified in plant workers less than 50 years old; an apparent 5-fold increase compared to similar control populations. Although an excess of prostate cancer cases was identified, the increase could largely be explained by the intensive prostate tumor antigen (PSA) testing in place at the facility during the course of the study (Delzell *et al.*, 2001; Hessel *et al.*, 2004). The study sample size; however, was insufficiently large to allow a clear determination as to whether all of the excess prostate cancer cases were in fact due to the intensive screening program.

In the large-scale prospective Agricultural Health Study a cohort of 55,332 male pesticide applicators in Iowa and North Carolina, Alavanja *et al.* (2003) reported that the OR for "ever using" atrazine in relation to prostate cancer incidence was 0.94 (95% CI=0.78 to 1.14). While the follow-up period was ill-defined due to the nature of the questionnaire used, and the exposure matrix of "ever" *versus* "never used" was rather crude, this large scale study provides no evidence to indicate that atrazine exposure is a risk factor for the development of prostate cancer.

Two studies, reported on by Mills and associates (Mills, 1998; Mills and Yang, 2003) have attempted to correlate 1993 pesticide usage data in relation to the incidence rates of certain cancers during the period of 1988 to 1992 in various California counties. Using a regression analysis incorporating county age- and race-adjusted cancer rates, Mills (1998) reported that there was a marginally significant correlation between atrazine usage and the incidence of prostate cancer in blacks. No significant correlations were reported for Asian, Hispanic, or white males. This study is difficult to assess since it suffers from aggregation bias common to ecological studies as exposures of individual subjects to atrazine were not evaluated.

In their second study, Mills and Yang (2003) reported the presence of a marginally significant correlation between high usage of simazine, as opposed to atrazine, and the incidence of prostate cancer among members of the United Farm Workers Union of America. As in their previous study; however, only poundage data were used as a surrogate of exposure, with no verification of exposures of any individuals. In addition, the county-specific poundage (use) data were not normalized to account for the number of farm workers in that county. As a result, the studies by Mills (1998) and Mills and Yang (2003) provide no evidence to indicate that atrazine, or simazine for that matter, is causally associated with the development of prostate cancer.

The epidemiology studies that have assessed atrazine exposure in relation to the risk for development of prostate cancer, as whole, do not provide evidence of a casual association.

4.4 Summary of the Epidemiology Data

Overall, *in toto*, the epidemiology data are at best inconsistent, with no clear strong associations between confirmed atrazine exposure and risk for cancer at any specific site. The results of the recent large-scale Agricultural Health Study cohort (Alavanja *et al.*, 2003) and the studies on employees known to be exposed to atrazine at a manufacturing facility (Delzell *et al.*, 2001; Hessel *et al.*, 2003) support the conclusion that the human epidemiology studies do not provide adequate data to suggest that atrazine has carcinogenic potential in humans. This conclusion is consistent with that of previous reviews of the epidemiology data conducted by IARC (1999), the U.S. EPA (2002) and 2 independent FIFRA Scientific Advisory Panels (SAP, 2000, 2003).

5.0 DISCUSSION AND CONCLUSIONS

The research conducted to assess the carcinogenic potential of atrazine is extensive and likely greater than that conducted for any other pesticide. For that matter, the body of data available on atrazine is greater than that available for most food additives, pharmaceuticals, or most any other industrial chemical.

The animal data clearly show that atrazine has an effect on the development of commonly occurring spontaneous mammary gland tumors in the female Sprague-Dawley rat. This represents a common tumor, at a single-site, and in a single sex. There is no other evidence of a carcinogenic effect of atrazine in experimental animals, including the F344 rat.

Atrazine is neither mutagenic or genotoxic. The mode-of-action of atrazine has been extensively documented and shown to involve attenuation of LH surge at the level of the hypothalamus in the female Sprague-Dawley rat leading to a persistent estrogenic state, with subsequent promotion of spontaneously occurring mammary gland tumors. This mode-of-action, due to the existence of significant differences in reproductive ageing patterns between the female Sprague-Dawley rat and humans, is considered of no relevance to humans. The available data also rule out alternate modes-of-action, including potential effects of atrazine on prolactin secretion and on the activity of aromatase enzymes involved in the conversion of androgens to estrogen.

Finally, an analysis of the older human epidemiology data shows inconsistent results with respect to associations of atrazine with site-specific cancers, in particular NHL. More recent data on prostate cancer, including results from an atrazine manufacturing study and the large-scale Agricultural Health Study cohort, provide no evidence of a causal association. An excess of prostate cancer cases in the manufacturing facility was largely explained by the implementation of an intensive PSA screening program during the course of the study. In summary, the human epidemiology studies do not provide any indication that atrazine is causally related to the development of any type of cancer.

With the above data evaluated and conclusions established, it must follow that atrazine be considered as "Not likely to be carcinogenic to humans". This conclusion echoes those of previous, and in general recent, reviews of the carcinogenic potential of atrazine (IARC, 1999; SAP, 2000, 2003; U.S. EPA, 2002, 2003; PMRA, 2004).

It is inconsistent, given the widespread scientific and regulatory consensus that atrazine is unlikely to be carcinogenic to humans, that NIEHS has at this time nominated atrazine for listing in the 12th RoC. The rationale given for nomination was the IARC (1999) review; however, this review does not conclude that atrazine is anticipated to be a human carcinogen. Rather, the IARC (1999) evaluation concluded quite the opposite on the basis of the mode-of-action data. In any case, given the apparent liberal rationale allowed for nomination of a chemical for listing or delisting in the RoC, it is worthwhile to compare the data on atrazine, and the associated conclusions of other scientific authorities, to the NTP's listing criteria for inclusion of a chemical in an RoC.

The criteria for listing of a chemical involve 2 separate categories "Known to be a human carcinogen" and "Reasonably anticipated to be a human carcinogen". The separate listing criteria for each of these classifications are:

> "Known to be a Human Carcinogen: There is sufficient evidence of carcinogenicity from studies in humans which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.
>
> Reasonably Anticipated to be a Human Carcinogen: There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias or confounding factors, could not adequately be excluded, or there is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor, or age at onset; or there is less than sufficient evidence of carcinogenicity in humans or laboratory animals, however; the agent, substance or mixture belongs to a well defined, structurally-related class of substances whose members are listed in a previous Report on Carcinogens as either a known to be human carcinogen or reasonably anticipated to be human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

In addition, the NTP criteria state that conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to mechanism of action data that may indicate an agent acts through mechanism(s) that does not operate in humans. Such chemicals would, therefore, not reasonably be anticipated to cause cancer in humans.

Clearly, the available human epidemiology data are insufficient to indicate a causal relationship between atrazine exposure and increased incidence and/or mortality due to cancer. At best the epidemiological data are inconsistent. As a result, there is no scientific basis to list atrazine in an RoC as "Known to be a human carcinogen". This classification quite rightly is assigned to chemicals such as vinyl chloride, asbestos, *etc.* for which compelling cause-and-effect relationships have been established.

With respect to listing as "Reasonably Anticipated to be a Human Carcinogen", the epidemiology data do not meet the criteria as "limited evidence of carcinogenicity in humans, which indicates that causal interpretations [are] credible...". As previously stated, the ecological epidemiology studies of farming populations have produced inconsistent results, where associations between atrazine and various cancers were weak, and could easily be explained by the presence of confounding variables or study bias. In fact, the U.S. EPA (2003) stated that "The Agency does not find any results among the available studies that would lead us to conclude that potential cancer risk is likely from exposure to atrazine". This conclusion echoes that of IARC (1999) who stated that there was *inadequate evidence* (*i.e.*, not *limited* or *sufficient evidence*) of the carcinogenicity of atrazine in humans. As a result, atrazine does not meet the first part of the criteria for listing in the RoC as "Reasonably Anticipated to be a Human Carcinogen".

The second part of the listing criteria for inclusion in an RoC as "Reasonably Anticipated to be a Human Carcinogen", refers to the strength of data from animal toxicology studies. Beyond an increase in the incidence of tumors, the criteria indicate that the data must also show one of: increases in multiple species, in multiple tissue sites, by multiple routes of exposure, or to an usual degree with regard to incidence site or type of tumor, or age at onset. While atrazine has been shown to support the earlier onset of common spontaneous mammary gland tumors in the female Sprague-Dawley rat, atrazine does not induce tumors in any other species, at any other anatomical site, or by any other route of exposure. Also, the tumor type associated with atrazine exposure in the female Sprague-Dawley rat is a common spontaneous neoplasm and hence is not usual to any significant degree. Based on this analysis, there would be appear to be no scientific basis to list atrazine on the basis of the animal toxicology data.

Beyond the strict interpretation of the listing criteria which itself provides no basis for the listing of atrazine, the NTP guidelines clearly indicate that scientific judgment and mechanism of action data are to be considered. Since the mechanism-of-action of atrazine (attenuation of the LH surge) is widely considered to be of no relevance to humans (IARC, 1999; SAP, 2000; U.S. EPA, 2002, 2003; PMRA, 2004), even the finding of promotion of mammary gland tumors in the female Sprague-Dawley rat can be discounted. As such then, there is no other toxicology data to indicate that atrazine has a carcinogenic effect. Therefore, the animal toxicology data provide no basis for the listing of atrazine in the RoC.

Not only do the animal toxicology data provide no evidence to support listing of atrazine in the RoC, they in fact would be sufficient to nominate atrazine for ***delisting*** should it have been listed in an RoC previously. The mechanism of action data would have been sufficient for delisting much the same as the rodent forestomach tumors were discounted in the delisting of ethyl acrylate and the bladder tumors were considered not relevant to humans in the delisting of saccharin in the 10th RoC.

In conclusion, the database on atrazine, including the animal toxicology and human epidemiology data, provide no substantive scientific basis for consideration of atrazine for listing in the 12th RoC.

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U.S. EPA. 2002. MEMORANDUM: SUBJECT: Atrazine. HED's Revised Human Health Risk Assessment for the Reregistration Eligibility Decision (RED) DP Barcodes: D272009, D281936, D281917. PC Code 080803. Case No. 0062. FROM: Catherine Eiden, Senior Scientist. Reregistration Branch 3, Health Effects Division (7509C). TO: Kimberly Lowe, Special Review Manager, Special Review and Reregistration Division (7508C). Office of Prevention, Pesticides, and Toxic Substances. U.S. Environmental Protection Agency; Washington, D.C. 20460. April 16, 2002.

U.S. EPA. 2003. MEMORANDUM: SUBJECT: Review of Atrazine Cancer Epidemiology DP Barcode D295200, Chemical #080803. FROM: Jerome Blondell, Ph.D.; Health Statistician Chemistry and Exposure Branch, Health Effects Division (7509C) and Vicki Dellarco, Ph.D.; Senior Science Advisor, Health Effects Division (7509C). Office of Prevention, Pesticides, and Toxic Substances. U.S. Environmental Protection Agency; Washington, D.C. 20460. October 28, 2003.

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